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Original research

Comparative evaluation of digital radiography, electronic apex locator and simultaneous working length determination on postoperative pain after root canal treatment: a randomized clinical trial

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Purpose
The study aimed to compare postoperative pain after root canal preparation using three different methods of working length determination.

Materials and Methods
60 patients diagnosed with symptomatic irreversible pulpitis were randomly divided into three groups based on the method of working length (WL) determination. Group 1: digital radiograph (DRG), Group 2: electronic apex locator (EAL), Group 3: the simultaneous working length control (SLC) method using an endomotor with an integrated apex locator. The root canal treatments were completed in a single visit, and patients were asked to record their pain response using the Visual Analog Scale (VAS) at 6, 24, 48, and 72 hours postoperatively.

Results
Group 1 (DRG) recorded the highest postoperative pain score, while the lowest was recorded by Group 3 (SLC). There was a statistically significant difference in the VAS pain scores between DRG and SLC ($p<0.05$) at 6-, 24- and 48-hour intervals.

Conclusion
Within the limitations of this study, it can be concluded that the SLC can be a helpful working length determination technique to reduce postoperative pain.

Keywords: *Electronic apex locator, postoperative pain, radiograph, root canal therapy, simultaneous working length control, visual analogue scale*

Introduction

The American Association of Endodontics has defined working length (WL) as "the distance from a coronal reference point to the point at which the canal preparation and filling should terminate" (1). Precise determination of WL is essential for the success of endodontic treatment, as both over and under-instrumentation can adversely impact the outcome. A WL calculated beyond the apical foramen can result in the extrusion of debris, irrigants, and root overfilling (2). This can intensify and prolong postoperative discomfort and reduce the odds of treatment success by 62% (3). Conversely, when the WL is short of the minor apical diameter, insufficient canal debridement and underfilling can occur, leading to an increased risk of apical periodontitis and decreased success rates. Short root canal fillings have 3.1% higher odds of being associated with apical periodontitis, and for every uninstrumented millimeter, there is a 12% reduction in the success of treatment (3,4). Conventionally, WL determina-

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tion has relied on radiographic methods. The technological advancement towards digital imaging has overcome several limitations of the traditional X-ray film radiographs. Digital imaging offers faster image processing with enhanced image quality, eliminates chemical processing, minimizes hazardous waste, requires less radiation than films via digital intraoral sensors and therefore reduces radiation exposure to the patient. Currently, there are two primary modes of digital intraoral imaging used in dentistry. The first is computed radiography, which employs photostimulable phosphor (PSP) plates to produce images. In contrast, direct digital radiography uses solid-state detectors, such as charge-coupled devices (CCDs) or complementary metal-oxide semiconductors (CMOS) systems. The digital detectors in essence changes how we acquire, store and display images. However, radiographic methods including digital dental radiography retains several limitations, including image distortion, exposure to ionising radiation, compromised image clarity due to the superimposition of anatomic structures and the incapacity to determine the location of apical constriction/ foramen, which may significantly vary from the radiographic apex (5).

The incorporation of electronic apex locators (EALs) with conventional radiography has significantly improved the precision and accuracy of working length (WL) determination. The number of radiographs required has also decreased as a result of this integration, lowering the radiation exposure to patients. Sunada developed the first apex locator which utilized direct current to determine the length of a root canal. The device was based on the principle that both the mucous membrane and the periodontium have uniform electrical resistance values. The initial generations of EAL had unreliable readings and poor accuracy in the presence of root canal irrigants or tissue fluids. The modern generation of EALs, such as the Propex Pixi apex locator, employs multiple frequency to measure change in impedance as the minor apical foramen is reached (6). These advanced apex locators are equipped with integrated microprocessors that can process using algorithm calculations the continuous change in impedance data as the files are advanced within canal. This enables the apex locators to determine the working length of a tooth with precision. Studies have reported an estimated accuracy in WL determination of 90% with modern electronic apex locator (7,8).

The simultaneous working length determination is a newer development that allows clinicians to clean and shape root canals while monitoring the file's position inside the canal using dynamic feedback from EAL. The Tri Auto ZX2 (J Morita Corp, Tokyo, Japan) is an endodontic motor with a built-in EAL that provides continuous feedback during root canal instrumentation and allows clinicians to make real-time adjustments to the WL. The motor has auto apical reverse and auto apical stop operation, which ensures that as soon as the tip of the file reaches the apical foramen, the motor reverses the file safely or stops rotating, reducing the risk of over- instrumentation (9).

Pain control is a major concern for endodontic patients and dentists. The frequency of postoperative pain occurrence ranges from 3% to 58% (10). Periradicular tissue irritation during root canal therapy triggers an acute inflammatory response, causing the release of chemical mediators and alterations in local adaptation and pressure in the periapical tissue (11). Precise determination of the WL can impact the occurrence of postoperative pain (12).

The present study aimed to compare the postoperative pain levels associated with three different WL measurement techniques: 1) Digital radiographic method (DRG), 2) EAL determination, and 3) Simultaneous length control using an endomotor with integrated Electronic Apex Locator (SLC). The null hypothesis was that there is no significant difference in the levels of postoperative pain associated with the three different WL measurement techniques in endodontic treatment.

Materials and Methods

Ethical approval

The present in-vivo study was conducted in the Department of Conservative Dentistry and Endodontics. It was approved by the ethics committee of the faculty of medicine and registered in the Clinical Trials Registry of India. (Reference id CTRI/2019/07/019960).

Sample size determination

The sample size calculation was done using Gpower software (Franz Faul, University of Kiel, Germany) with an effect size of 0.82, alpha error =0.05 and power of 0.8.

Study design and patient selection

This study was designed as a parallel-group, randomized clinical trial with three arms, each with an equal allocation ratio of 1:1:1 ratio. A total of 60 patients (33 men and 27 women) were enrolled for this in-vivo study after obtaining a written informed consent. The study subjects were recruited from a pool of patients referred to the Department of Endodontics between July 2019 to June 2020. Only single-rooted teeth were taken into consideration for the study. Healthy patients having symptomatic irreversible pulpitis and without any periapical lesion or any systemic disease were included in this study. Patients with systemic diseases, swelling, sinus tract, severe periodontal disease or resorption, history of bruxism clenching or previously initiated or completed root canal treatment requiring retreatment were excluded. An Intraoral periapical radiograph was used to confirm the presence of a single root. The clinical history of lingering thermal, spontaneous or referred pain suggestive of symptomatic irreversible pulpitis was confirmed by pulp sensibility test by cold refrigerant spray (Endo Ice, Hygienic Corporation, Akron, OH) and electric pulp test (Gentle Pulse, Parkell, New York, USA). After the endodontic access, the diagnosis was confirmed by the presence of vital bleeding pulp tissue.

Root canal preparation

The maxillary teeth were anesthetized using a local infiltration technique, whereas the mandibular teeth were anesthetized by inferior alveolar nerve block supplemented with buccal infiltration or mental nerve block. The anes-

thetic solution used was Lidocaine with 1:80000 adrenaline (Lignox 2%, Indoco Remedies, India). A rubber dam was placed, and an access cavity was prepared with the help of Endo-access burs (Dentsply, USA). Size #10K and #15K hand (Mani Inc, Japan) files were passively inserted into coronal two-thirds of a root canal as pathfinding files. Coronal flaring was done using the Protaper Gold shaper files (S1, S2, SX). Working length was estimated as soon as the 15 K hand file appeared to reach and bind at the tentative working length. The tentative working length was established by measuring the radiographic length of the tooth on a diagnostic radiographic digital image acquired by paralleling technique and subtracting a safety factor of 2mm to ensure that instruments would not be extended beyond the apical foramen. Based on the method of working length determination, patients were randomly assigned into three groups. Block randomization with a block size of 6 patients (with each block containing two patients per treatment arm) was done using software (available on www.randomizer.org). Random sequence generation was done by a person not involved in the study and revealed to the clinician at the time of the procedure. The three experimental groups assigned were:

Group 1 Digital Radiographic method (DRG): In this group, the WL of the canal was established by digital radiograph (Sopix^2, Acteon) using Weine's method by subtracting 0.5mm from the distance measured from the radiographic apex. The images were acquired by a long cone paralleling technique using a positioning device (Rinn XCP-ORA, Dentsply Sirona). Digital radiography was done using a CMOS sensor (Sopix2 Acteon) with a 25 pl/mm resolution. The root canal preparation was done by Protaper Gold using F3 as the final file with NSK Endomate DT endomotor(NSK, Japan) as per the manufacturer's instruction.

Group 2 Electronic apex locator method (EAL): In this group, the WL was established by using Propex Pixi (Dentsply, USA) apex locator following the manufacturer's instructions. The lip clip was placed in the mouth, and the file clip was attached to the 15 K file. The file was advanced in the root canal till the 00 reading was obtained in the Propex Pixi apex locator. The rubber stopper was adjusted to a coronal reference point. The file was removed from the canal, and the distance between the rubber stopper and the tip of the file was measured on the endodontic ruler (Dentsply, USA). 0.5mm was subtracted from the value to obtain the final WL. The root canal was prepared with a Protaper Gold system up to size F3 thourough NSK Endomate DT (NSK, Japan) endomotor according to the manufacturer's instructions.

Group 3 Simultaneous length control using endomotor with integrated Electronic Apex Locator (SLC). The preparation in this group was done using Tri Auto ZX2 (J Morita, Japan) endomotor, which has a built-in apex locator. The mode was set as an auto-apical stop to ensure that there was no over-preparation during instrumentation. Preparation was done up to Protaper Gold size F3.

For all the groups, in between each file used, the canals were copiously irrigated with 5.25% sodium hypochlorite (Prime Dental, India). Flutes of the files were cleaned with wet gauze after each instrumentation, signs of distortion or wear of the file were checked, and apical patency of the root canal was maintained with a #10K file. Following completion of the biomechanical preparation, a radiograph was taken after placing a 6% size 30 master cone gutta percha (Meta Biomed, Korea) to the working length. The canals were obturated using epoxy resin sealer (AH Plus, Dentsply Maillefer) and cold lateral compaction of the gutta-percha. The entire treatment was performed by a single operator (post-graduate endodontic resident) as a single visit endodontic procedure. Ibuprofen 400 mg was prescribed to the patients, with instructions to use it as a rescue analgesic only in the event of unbearable pain.

Pain evaluation

Patients were provided with a questionnaire for recording the postoperative pain intensity and analgesic intake at 6, 24, 48 and 72 hours. They were instructed on how to use a VAS (Visual Analog Scale) to rate their pain and document their responses in the questionnaire. Furthermore, before each time interval, patients were reminded via a phone call and an electronic message to submit their response.

Statistical analysis

The data were assessed for homogeneity by the homogeneity of variance and normality by the Shapiro-Wilk test. Data on gender and dental arch and analgesic intake after procedure was evaluated by the $\chi 2$ test, while data on age and preoperative pain was analyzed by the One-Way ANOVA test. The postoperative pain scores were analyzed statistically using the Kruskal Wallis and Mann-Whitney U test, and Wilcoxon signed ranks test at a significance level of $p < 0.05$. All statistical analysis was conducted in a blind manner at a confidence interval of 95% (p=.05) and performed using SPSS 20.0 software (IBM Corp, Armonk, NY, USA).

Results

From the total sample of 60 patients, 33 (55%) were men, and 27(45%) were women. Each patient had only one tooth included in the study, making a total sample of 60 patients with 60 teeth. Out of the total teeth that were treated, 41 (68.3%) of them were maxillary teeth comprising 32 (53.3%) incisors and 9 (15%) premolars. 19 (31.7%) of them were mandibular teeth consisting of 11 (18.3%) incisors and 8 (13.3%) premolars. Two patients (one from the DRG group and one from the SLC group) did not respond and were lost during the follow-up. The mean age of the patients was 24.4 ±8.45, ranging from 16-52 years. The characteristics of the patient and the demographic data are shown in Table 1. The demographic data among the three groups were found to have no statistically significant difference among them.

All the methods of working length evaluation resulted in postoperative pain. The highest VAS pain scores for all three experimental groups were recorded at 6 hours postoperatively, and these scores decreased gradually over the 72-hour observation period (Figure 1). Intra-group comparison by Wilcoxon signed ranked tests showed that statistically significant differences existed within a group at 6, 24, 48 and 72 hours postoperatively for all the groups ($p<0.05$). The mean postoperative pain values evaluated after 6, 24, 48, and 72 hours are shown in Table 2. There was no significant difference in postoperative pain between males and females in any group at 6, 24, 48 and 72 hours.

Table 1: *Demographic data and preoperative pain levels*

Demographic data	Digital Radiograph	Electronic Apex Locator	Simultaneous Length Control	P value
Ageφ	24±8.2	23±10.1	26±7.8	p>0.05
Gender χ				
Male	11	13	12	p>0.05
Female	9	7	8	
Dental Archχ				
Maxillary	15	12	14	p>0.05
Mandibular	5	8	6	
Mean preoperative pain scoreφ	7.8±0.85	7.9±0.97	7.6±0.93	p>0.05

Φ: one way ANOVA test χ: χ2(chi square test)

Table 2: *Mean postoperative Pain at 6, 24, 48 and 72 hours and rescue medications needed in each group*

	Mean Postoperative Pain VAS scale (cm)				No. of patients taking analgesics
	6 hours	24 hours	48 hours	72 hours	
DRG	3.76[a]	2.59[a]	1.86[a]	0.71[a]	8[b]
EAL	3.12[ab]	2.06[ab]	1.71[a]	0.50[a]	3[a]
SLC	2.24[b]	1.51[b]	0.42[b]	0.34[a]	2[a]

The postoperative pain scores were analyzed statistically using the Kruskal Wallis test and Mann-Whitney U and Wilcoxon signed ranks test at a significance level of p <0 .05. Same superscript in a column indicates no significant difference, DRG= Radiographic method , EAL= Electronic Apex Locator , SLC= Simultaneous Length Control

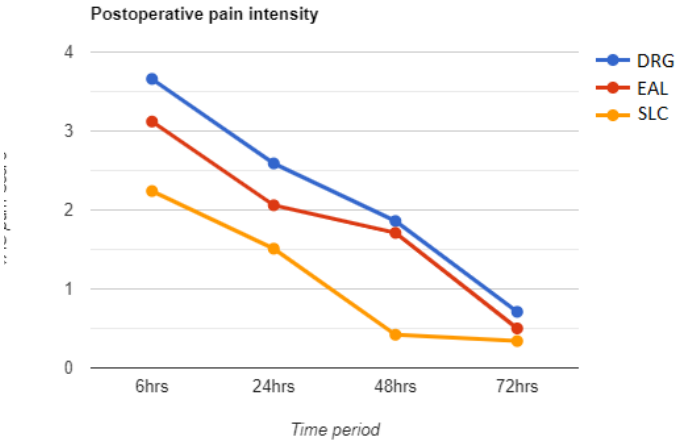

Figure 1. *Line graph illustrating the VAS pain scores over time in the three groups (DRG, EAL, SLC) DRG= Radiographic method, EAL= Electronic Apex Locator, SLC= Simultaneous Length Control.*

In this study, the SLC group (Group 3) experienced the least postoperative pain compared to all other experimental groups. The SLC group recorded the lowest VAS scores at all observed time intervals (6, 24, 48, and 72 hours). The study found a significant difference in VAS pain scores at 6, 24, and 48-hour intervals between Group 1 (DRG) and Group 3 (SLC) (p<0.05). Additionally, a significant difference in VAS pain scores was observed between Group 2 (EAL) and Group 3 (SLC) at the 48-hour interval. No statistically significant difference in postoperative pain levels existed between Groups 1 (DRG), Group 2 (EAL) and Group 3 (SLC) at the 72hour observation interval. 13 patients overall, with eight patients from Group1 (DRG), three patients from Group 2 (EAL) and two patients from Group 3 (SLC) required postoperative analgesics. There was a statistically significant difference in the postoperative analgesic intake of DRG (Group 1) compared to EAL (group 2) and SLC (group 3) (p<0.05).

Discussion

During endodontic treatment, clinicians primarily rely on radiographs and electronic apex locators for determining the working length. A recent innovation is the introduction of endodontic motors with integrated electronic apex locator, which simultaneously prepares and monitors the WL. There are very limited studies assessing the accuracy of the simultaneous working length determination. The present study was therefore conducted to evaluate and compare the effect of Digital radiography, electronic apex locator and dynamic working length measurement on postoperative pain. The present study found a statistically significant difference in the pain levels between the Group 1 (DRG) and Group 3 (SLC) at 6, 24, and 48 hours postoperatively. Thus, the null hypothesis of no difference in the postoperative pain levels between the experimental working length determination groups was rejected.

Radiographs are essential in endodontics during diagnosis, treatment and postoperative evaluation. However, radiographic images are a two-dimensional view of a three-dimensional object. To overcome this limitation, the present study employed a horizontal cone shift technique by taking two radiographic images of the same tooth at different horizontal angles and used the SLOB (same lingual opposite buccal) principle to interpret the images. These methods were utilized to attain an understanding of the tooth's three-dimensional anatomy and allowed for the verification

of the diagnosis of single-rooted teeth that were included in the study. Clinicians frequently rely solely on radiographic images to estimate the working length during root canal treatment. In order to prevent magnification errors that can result from incorrect angulation of the X-ray beam to the sensors, a long cone paralleling technique with a positioning device (such as the Rinn XCP-ORA from Dentsply Sirona) was implemented in this study. Digital radiography using CMOS (Complementary Metal Oxide Semiconductor) sensors was used in the study to acquire the images. Direct digital radiography systems for dental imaging use CMOS and CCD sensors. Compared to CMOS sensors, which are more recently available on the market, CCD sensors are well-established in the industry and have been used for many years. CCD sensors are known for producing images of high quality and low noise. A scintillator layer absorbs light in a CCD sensor before emitting photons that are captured by a photoconductive layer. The photoconductive layer then generates electrical charges that are read out by the CCD chip. On the other hand, CMOS sensors capture and amplify the electrical charges produced by X-ray energy using an array of tiny transistors. Unlike CCD, each pixel in a CMOS sensor has its own amplifier circuit, making the sensor more power-efficient and faster. Comparative studies have reported the quality of images made by the CCD and CMOS intraoral X-ray detectors to be similar (13, 14). However, there is possible cost saving and decreased power requirements associated with the adoption of CMOS technology.

In the present study, among all the experimental groups, radiographic determination of working length was associated with the highest recorded VAS pain scores at all the observed time intervals (6,24,48, and 72 hours). Additionally, a significantly higher number of patients in Group 1 (DRG) required rescue analgesic medications compared to Group 2 (EAL) and Group 3 (SLC). The radiographic method of WL determination used in the present study involves an arbitrary estimation of the apical foramen by subtracting a predetermined length from the radiographic apex. However, the variation in the position of the radiographic apex and the actual apical foramen is a primary cause of inaccuracy in radiographic WL determination, which may explain the higher postoperative pain scores observed in this group of patients (15).

Electronic apex locators use electrical measurements to precisely locate the apical constriction for working length determination, unlike the radiographic method that relies on an arbitrary estimation. Modern apex locators, like Propex Pixi, measure the changes in electrical impedance at two different frequencies, allowing for accurate and reliable measurements even in the presence of blood, pus, or other materials (16). In the present study, it was observed that the group using electronic apex locators had lower median pain scores on the visual analogue scale (VAS) at all the intervals measured (6, 24, 48, and 72 hours) compared to the group that used radiographic working length determination. However, the difference in the postoperative pain scores between the two groups was not statistically significant. One possible reason for potential errors in working length determination using an electronic apex locator is that the process involves manually measuring and transferring the working length to an endodontic file by marking it with a rubber stop. These manual techniques can introduce inaccuracies, as the rubber stop may be incorrectly placed or move during use, and the visual estimation may not be precise. These errors could impact the accuracy of the procedure in clinical practice. The results were consistent with the study by Tuncer and Gerek (17), where they reported no significant difference in the severity of postoperative pain between working length determination by digital radiography and electronic apex locator.

The integrated endomotor Triauto ZX 2 enables simultaneous instrumentation and working length control. These motors are capable of detecting and stopping at the working length, which minimizes the risk of over-instrumentation beyond the apex. This feature can help reduce periapical tissue damage and postoperative pain. This was confirmed in our research as the simultaneous working length determination (Group 3) resulted in the least amount of pain which was significantly lesser than the radiographic group (Group 1) at 6, 24 and 48 hours ($p<0.05$). The findings of this study are consistent with the results obtained by Arslan *et al*. (18), who found that the group that underwent simultaneous length control during root canal preparation had lower postoperative pain levels on day 1 than the control group in which working length and instrumentation were accomplished separately.

The present study found that the highest VAS pain scores were consistently recorded at 6 hours postoperatively, and these scores decreased gradually over the 72-hour observation in all the experimental groups. The results were statistically significant when intragroup comparison across the observed time intervals were made in all the experimental groups. These findings are consistent with the findings of several other studies (17,19,20). The most likely explanation for the higher pain scores observed at the 6-hour interval is that the patient's anesthesia fully wears off by this time, and the patient starts to experience pain. The allodynic and hyperalgesic pain responses are elicited by the nerve endings sensitized by the acute inflammatory mediators. However, the initial acute inflammation gradually decreases over a few days, and as a result, the pain reduces. Most patients in this study experienced little to no pain after 48 and 72 hours of treatment, and there was no significant difference in pain levels between the groups after 72 hours. This aligns with the findings of a prior study, which showed that pain severity tends to decrease over the course of several days, often decreasing by half after just one day (21). Tuncer and Gerek (17) reported minor to no pain within 12-48 hours of treatment, with pain decreasing to 22.9% and 27.3% within 24-48 hours.

Factors such as preoperative diagnosis and instrumentation techniques used during root canal treatment influence post-endodontic pain. In the present study, single rooted teeth with irreversible pulpitis were included to allow consistency in completing the biomechanical preparation and obturation in a single visit. A recently conducted systematic review found that after a root canal treatment was done in a single visit, there is a lower frequency of short-term post-obturation pain than performing a multiple-visit root canal treatment, without any significance in the rate of healing (22). All the procedures were performed by a single operator (post graduate resident) to offset interoperator variations in results obtained. Crown down rotary instrumentation was

utilized for the biomechanical preparation of the root canal system, as research has shown that it results in significantly lower debris extrusion compared to manual step back preparation (23,24). Coronal flaring is crucial in determining the appropriate initial apical size, as demonstrated by Pecora *et al.*, who found that pre-flaring significantly enhanced the precision of Root ZX electronic apex locator measurements, regardless of the file used (25, 26). Therefore, in the present study, coronal flaring was performed prior to determining the working length.

In the present study, visual analog scale (VAS) was used to measure the pain intensity which has been used previously in several studies to measure post-endodontic treatment pain (27,28). The VAS scale was the choice in this study because of the following reasons 1) It has a continuous scale which allows patients to indicate their pain intensity with high sensitivity 2) The quantitative measurements obtained by VAS pain scale is helpful in the statistical analysis and result interpretation 3) It is easy for the patient to understand and use.

The visual analog scale used in this study relies on the patient's subjective perception of pain, which can result in variability in scoring. The study participants were limited to single-rooted teeth with irreversible pulpitis, and therefore, the findings may not be applicable to more complex clinical situations, such as multirooted teeth, severe root curvatures, pulp necrosis, or periapical lesions. Future research should explore postoperative pain outcomes in these situations to ensure broader generalizability of the results.

Conclusion

Within the limitations of this study, it can be concluded that the SLC technique resulted in significantly lower postoperative pain after 6, 24 and 48 hours, than the DRG method, and could be a beneficial non-pharmacological method to reduce postoperative pain. The postoperative pain levels in the 72-hour assessment period were reduced to minimal or no discomfort level irrespective of the WL determination technique used.

Türkçe özet: *Kök kanal tedavisinden sonra postoperatif ağrıda dijital radyografi, elektronik apeks bulucu ve eşzamanlı çalışma uzunluğu belirlemenin karşılaştırmalı değerlendirmesi: randomize bir klinik çalışma Amaç: Çalışma, kök kanal preparasyonundan sonra postoperatif ağrıyı üç farklı çalışma uzunluğu belirleme yöntemi kullanarak karşılaştırmayı amaçladı. Gereç ve Yöntem: Semptomatik irreversibl pulpitis tanısı alan 60 hasta, çalışma boyu (WL) belirleme yöntemine göre rastgele üç gruba ayrıldı. Grup 1: dijital radyografi (DRG), Grup 2: elektronik apeks bulucu (EAL), Grup 3: entegre bir apeks bulucu ile bir endomotor kullanan eş zamanlı çalışma uzunluğu kontrolü (SLC) yöntemi. Kök kanal tedavileri tek seansta tamamlandı ve hastalardan postoperatif 6, 24, 48 ve 72. saatlerde Visual Analog Skala (VAS) ile ağrı yanıtlarını kaydetmeleri istendi. Bulgular: Postoperatif ağrı skoru en yüksek Grup 1'de (DRG), en düşük ise Grup 3'te (SLC) kaydedildi. DRG ve SLC arasında 6, 24 ve 48 saatlik aralıklarla VAS ağrı skorlarında istatistiksel olarak anlamlı fark vardı (p<0,05). Sonuç: Bu çalışmanın sınırlamaları dahilinde, SLC'nin postoperatif ağrıyı azaltmak için yararlı bir çalışma uzunluğu belirleme tekniği olabileceği sonucuna varılabilir. Anahtar Kelimeler: elektronik apeks bulucu; ameliyat sonrası ağrı; radyografi; Kök kanal tedavisi; eş-zamanlı çalışma uzunluğu kontrolü; görsel analog Ölçeği*

Ethics Committee Approval: The study protocol was approved by the ethics committee of the faculty of medicine and registered in the Clinical Trials Registry of India. (Reference id CTRI/2019/07/019960).

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

Author contributions: BS, SA, SKM participated in designing the study. BS participated in generating the data for the study. BS, SA, DL participated in gathering the data for the study. BS, SA, DL participated in the analysis of the data. BS, SA wrote the majority of the original draft of the paper. BS, SA participated in writing the paper. DL, SKM has had access to all of the raw data of the study. BS, SA, DL, SKM has reviewed the pertinent raw data on which the results and conclusions of this study are based. BS, SA, DL, SKM have approved the final version of this paper. BS, SA, DL, SKM guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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